Technical Bulletin No. 788 • January 1942

UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

Relative Effectiveness of Acid Lead Arsenate and Other Materials as Stomach Poisons for the Larvae of the Japanese Beetle[1]

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INTRODUCTION

Preliminary experiments by Leach (*17*)[3] in 1922 demonstrated that larvae of the Japanese beetle (*Popillia japonica* Newm.) could be killed by adding certain arsenicals to the soil. Since that time many different materials have been tested to determine their value as stomach poisons for use against this insect in the soil. The information presented in this bulletin covers the writer's investigation from 1929 to 1936, inclusive, and gives the results obtained with different arsenicals, fluorine compounds, borates, derris, pyrethrum, and other miscellaneous materials.

Larvae of the Japanese beetle are killed when they ingest poisonous materials while burrowing through soil or feeding on roots growing in this soil. When a larva consumes a lethal quantity of poison, it ceases feeding and burrowing, discharges the contents of the alimentary tract, becomes flabby, and eventually dies. The action of a stomach poison in the soil is complex, being influenced by the development, activity, and susceptibility of the larvae, the nature and concentration of the material, and the physical and chemical characteristics of

[1] Received for publication May 16, 1941.

[2] The writer wishes to acknowledge with deep appreciation the work of the late Francis E. Baker, assistant entomologist, who assisted in the routine work of conducting the biological tests described in this bulletin.

[3] Italic numbers in parentheses refer to Literature Cited, p. 31.

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the soil. Newly hatched larvae are more readily killed than fully developed third instars. Larvae in soil containing an abundance of roots are more easily killed than those in fallow soil. Larvae succumb more quickly at high than at low temperatures; when the temperature of the soil is below 50° F. the larvae are practically inactive and are usually not seriously affected by a stomach poison.

In a recent study of the insecticidal action of acid lead arsenate in different soils from New Jersey (*13*) it was found that the coefficient of effectiveness of the material ranged from 0.30 in Colts Neck loam to 2.18 in Lakewood sand. A significant correlation was found between the insecticidal effectiveness of acid lead arsenate and the soluble constituents of the soils. The concentration of soluble phosphates, calcium, and magnesium appeared to be the most important factors influencing the insecticidal action. The greater the concentration of soluble phosphates and calcium in the soil, the more effective was the arsenical treatment, and the greater the concentration of soluble magnesium, the less effective it was. The influence of soluble chlorides, nitrates, ammonia, potassium, and manganese was of minor importance. It was also found that the insecticidal action of acid lead arsenate in a soil could be modified by adding different phosphates.

It is very desirable, in a search for new soil insecticides, to attempt to control as many as possible of these variable factors. The variation produced by differences in the type of soil, the temperature, moisture and food can be reduced to a minimum by conducting the preliminary experiments under controlled conditions with one type of soil. The soil used for all the experiments in this study was a sassafras sandy loam. Unfortunately, the larvae vary in their susceptibility to a stomach poison, even under controlled conditions, depending upon the season of the year, the source of the test insects, and the period of time elapsing between digging them in the field and using them in the experiments.

In the search for new insecticides the determination of the relative effectiveness of many materials is more important than isolated determinations on the action of individual compounds. The comparative effectiveness of acid lead arsenate, which has become established as a standard soil stomach poison for the control of larvae of the Japanese beetle, and another material is of more value than the exact measurement of the relation between the dosage and the mortality produced by this material. When acid lead arsenate is used as a standard in each individual test, as in these, the variation in the susceptibility of different lots of larvae is not a limiting factor because it does not modify appreciably the comparative value of a material as a soil insecticide.

CONDITIONS FOR TESTING STOMACH POISONS

It has been observed that when the temperature of the soil is below 50° F. the larvae of the Japanese beetle are largely inactive and are affected by a stomach poison only to a limited extent. As the temperature of the soil is raised to 85° the activity of the larvae increases and the time required for an insecticide to become effective is progressively decreased. At a temperature of 80° to 85° a stomach poison may be effective in 2 weeks, whereas at 55° to 60° from 6 to 8 weeks may be required. Temperatures above 85° appear to retard the activity of the larvae. Activity is also impaired in soils that are excessively wet or dry.

The laboratory tests with stomach poisons were conducted in 5-inch earthen pots in the controlled chambers shown diagrammatically in figure 1. The walls and ceilings of two cellars were lined with insulating board and plastered. The temperature was controlled by a commercial thermostat adjusted to keep the temperature between 80° and 85° F. The thermostat activated a centrifugal pump which forced water at 160° through a heating unit and started a fan which drew air from the chamber, passed it over the heating unit, and returned it to the chamber. Fans were also placed in the aisles to

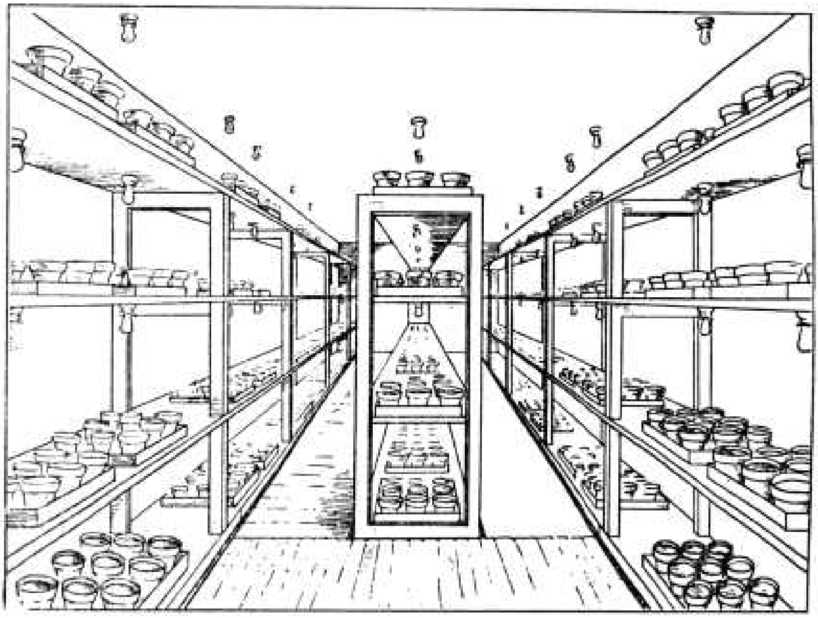

FIGURE 1.—Diagram of a chamber used in testing materials as soil stomach poisons against larvae of the Japanese beetle.

circulate air in the chamber. The pots were plunged in moist peat to conserve the moisture and to make necessary the application of water to the soil only at infrequent intervals.

PROCEDURE FOR CONDUCTING THE TESTS

The procedure for conducting a test to determine the value of a material as a stomach poison against larvae of the Japanese beetle is fundamentally the same as that used in the biological assay of drugs and disinfectants, in that the mortality produced by different concentrations of the test material and the standard are determined experimentally under like conditions. If the stock of test larvae were constant in its average susceptibility to the standard, the results obtained with a test material could be compared with a single dosage-mortality curve of comparatively high accuracy, determined from many experiments with the standard. As the population of larvae dug in the field is not stable in this respect, however, each separate assay of a material must be based on parallel tests between the standard and the test material.

In conducting each test, the standard (acid lead arsenate) was mixed with moist, sifted sassafras sandy loam at rates equivalent to applying 250, 500, 1,000, 1,500, and 2,000 pounds per acre and mixing to a depth of 3 inches. Prior to 1935, 0.735 cubic foot of soil, sufficient to fill twenty 5-inch earthen pots, was first spread in a thin layer on a smooth concrete floor and then the required quantity of the lead arsenate was scattered uniformly over the surface and mixed with it by raking and shovelling until uniformly dispersed. Later, in order to enhance the dependability of the data, the quantity of soil was doubled, sufficient to fill forty 5-inch pots, and was mixed more rapidly and uniformly with the poison by passing it three times through a gyratory riddle.

After the drainage holes of the pots had been covered with 16-mesh wire to prevent the subsequent escape of larvae, they were filled with the treated soil. An equal number of pots were filled with untreated soil for the determination of the natural mortality of the insect. The test materials were then applied in the same manner, or pots of soil that had been treated previously and exposed to weathering in the field were brought in and prepared for testing by removing the soil from each group of pots and passing it through the gyratory riddle to remove all larvae naturally infesting the pots and to remix and loosen the soil. Rye was sown in each pot to supply an abundance of food for the larvae.

The pots were plunged to a depth of 2 inches in moist peat in the constant-temperature chamber. After 48 to 72 hours, when the soil was at the proper temperature and the grain had sprouted, five active third instars were placed in each pot. The most satisfactory procedure found for infesting the pots was to make five holes 2 inches deep with a small dibble, place one larva in each hole, and fill the holes with soil. Third instars were selected for test purposes because larvae in this instar are most resistant to insecticides, are more easily handled without fear of injuring them, and are more readily seen in the soil during examination than the other instars. It was not feasible, because of cannibalism, to put more than five larvae in a 5-inch pot.

The soil was removed from each pot 14 days after the larvae had been introduced, and the number of dead and living larvae were recorded. Only larvae which were in the various stages of decomposition were classified as killed by the treatment. All other larvae, although in some cases showing evidence of injury, were classified as surviving. When the examination was completed, all series of pots of the test materials which produced promising results were plunged in the field to determine subsequently the influence of weathering on the insecticidal action. The soil containing the acid lead arsenate was discarded, and the pots were thoroughly washed before they were used for other tests.

ANALYSIS OF DATA

It will be observed in the tabulation of the data that some larvae usually survived at the end of 14 days even in soil with the highest concentration of the standard and other materials. This should not be interpreted as being indicative of the maximum killing power of a treatment because the progress of the insecticidal action was deliberately interrupted before the mortality approached 100 percent in the median concentrations in order that the assay of the materials might be made more accurately. The dosage-mortality curves of the stomach poisons on larvae of the Japanese beetle are typically sigmoid in character. With a relationship of this nature, the change in percentage of mortality is the smallest per unit of dosage near mortalities of 0 and 100 and the largest near 50 percent.

It has been established by Strand (*25*), Tattersfield and Morris (*28*), Trevan (*29*), and other investigators that the closest agreement in insecticidal tests occurs when the concentration causing 50 percent mortality is selected as the criterion for comparisons. A method of expressing the relative effectiveness of stomach poisons on the larvae of the Japanese beetle has been developed (*11*) by use of the median effective concentrations of the standard and the test material. By this procedure the concentrations producing median survivals in the test material and the lead arsenate with their respective standard errors were calculated, and the relative effectiveness of a test material was expressed as a coefficient of the standard. The significance of the differences between the coefficients of the various materials could be determined from a consideration of their respective standard errors.

The precision of the results obtained at the 50-percent point is often of distinct advantage in the study of the factors which influence insecticidal action. Unfortunately no simple relationship exists between the median effective concentration and that required to kill 100 percent. For example, 50 percent of the population may be killed with 750 pounds of the standard and 100 pounds of a test material, 75 percent with 1,000 and 200 pounds, and 90 percent with 1,200 and 1,000 pounds, respectively. In this case the two dosage-mortality curves, one from the test material and the other from the standard, are converging as they approach 100 percent, but it is not uncommon to have curves which are close at the 50-percent point become sharply divergent as they approach 100-percent mortality. Therefore, even though the concentration of the standard necessary to assure 100-percent mortality of the larvae in the field is known, the quantity of the test material required to produce equivalent results cannot be estimated from the coefficient derived at the 50-percent point.

As it is believed that the results obtained from a laboratory assay of a material are of more practical value when expressed in terms of pounds per acre thereof that can be expected to produce 100-percent mortality in the field, an attempt was made to determine accurately the 100-percent mortality points for the standard and test materials so that the coefficient of effectiveness at this point might be derived. It was found that the concentration of a material just sufficient to cause complete mortality could not be determined very precisely experimentally because of the nature of the toxicity curves. The calculation of this point from other points on the sigmoid curve was also disappointing. Shepard (*23*) adapted the formula used by Clark (*7*) to the dosage-mortality curves of fumigants against the rice weevil (*Sitophilus oryza* (L.)) and apparently was able to estimate the maximum "nonlethal" dose with a high degree of accuracy.

When this formula was used to estimate the minimum concentration of a stomach poison in the soil necessary to kill practically 100 percent of the larvae of the Japanese beetle, the estimated quantity was often so far in excess of, or below, that at which complete mortality occurred experimentally as to make the estimated figure of doubtful value.

It is possible that the method suggested by Bliss (*2*, *3*) for the conversion of the sigmoid curve into a straight line might be used to estimate the dosages approaching those necessary for complete mortality; but another procedure, which will be described presently, appeared to be satisfactory for estimating the rates at which the different test materials would have to be used to be effective against the larvae.

It has been determined experimentally under field conditions that an application of acid lead arsenate at the rate of 1,000 pounds per acre, very thoroughly and intimately mixed with the upper 3 inches of a sassafras sandy loam before the eggs hatch, will cause complete mortality of the larvae. It seemed proper, therefore, to evaluate in the laboratory the comparative effectiveness of the different materials by estimating the rates at which they would have to be applied to be equivalent in insecticidal value to 1,000 pounds of acid lead arsenate, using third instars as test insects under the conditions and according to the procedure previously described.

In each series of tests there are usually four independent causes of variation in the larval survival, (1) the chemical composition of the materials, (2) the rate of application, (3) the period the materials have been in the soil, and (4) the random or experimental error. The analysis of variance has been found to be the most satisfactory procedure for determining the significance of the differences in larval survival caused by the variation in chemical composition, rate of application, and the period in the soil. When this procedure demonstrated that there was no significant difference in insecticidal value between the standard and a test material there was no necessity for analyzing the data further, but when significant differences were demonstrated the data were analyzed to a greater extent. In the latter case the rate at which a test material would have to be applied to be equivalent in insecticidal value to 1,000 pounds of freshly applied acid lead arsenate was estimated by interpolation of the larval survivals, using the formula $A+\frac{(B-A)\ (C-E)}{(C-D)}$, where A is the number of pounds per acre of the test material giving a survival just above that for 1,000 pounds of the standard, B is the number of pounds per acre of the test material giving a survival just below that of the standard, C is the survival with treatment A, D is the survival with treatment B, and E is the survival with the 1,000-pound level of the standard. The standard error of this value is that of the quotient $\frac{C-E}{C-D}$, multiplied by the constant $(B-A)$. The standard errors of differences $(C-E)$ and $(C-D)$ were known from the analysis of variance. The error of the treatment with a test material estimated to be equivalent to the standard was computed by the formula

$$\frac{(B-A)\ (C-E)}{C-D}\sqrt{\frac{\sigma^2_{C-E}}{(C-E)^2}+\frac{\sigma^2_{C-D}}{(C-D)^2}}.$$

The final estimate of the effectiveness of a material in comparison with the 1,000-pound standard was made at the end of 14 days under conditions in which the temperature was maintained between 80° and 85° F. It is realized that with the more rapidly acting materials most of the effect might have been obtained in a shorter period. Materials which had had little toxic action on the larvae by the end of this period might have shown some value if the exposure had been prolonged. It is known, however, that the results obtained under the conditions of the tests are very similar to those obtained with third instars in the field after a period of 6 to 8 weeks when treatments are applied between September 15 and October 1. As the activity of larvae in the field after November 15 is at a low level because of the low temperatures, stomach poisons at this time have little effect on them. It may be considered, therefore, that the evaluation of the material at the end of 14 days in the laboratory is what might be expected at the end of the active season in the fall. Any material which was definitely inferior to acid lead arsenate at the end of the test period probably would have little value for the control of the larvae. Only materials which were equal to or more effective than acid lead arsenate at the end of the test period can be considered as possible substitutes.

ACID LEAD ARSENATE AS A STOMACH POISON

Before the various materials are considered as substitutes for acid lead arsenate, some attention should be given to this chemical as a stomach poison in the soil, particularly as to the influence of some of its physical and chemical properties and the persistence of the insecticidal action in the soil.

Influence of the Size of Particles on Insecticidal Action in the Soil

Through the cooperation of a commercial grinding company, R. D. Chisholm, of the Division of Insecticide Investigations of the Bureau of Entomology and Plant Quarantine, reground a commercial sample of acid lead arsenate in order that the influence of the size of the particles on the effectiveness of the material in poisoning larvae of the Japanese beetle in the soil might be investigated. The original sample was analyzed and found to be of the following composition: Total arsenic oxide (As_2O_5) 31.44 percent, total lead oxide (PbO) 64.21 percent, water-soluble arsenic oxide 0.40 percent, and water 0.37 percent. Three grindings were made with the material alone at different speeds, and one was made with the material to which 2 percent of 200-mesh gum arabic had been added previously.

The original material and the different batches of the reground material were analyzed by L. D. Goodhue, also of the Division of Insecticide Investigations, to determine the size of the particles in each of them. Each sample was suspended in water with gum arabic and placed in a differential manometer-type sedimentation apparatus. The density of the dispersed substance was determined at intervals. From the relation between the velocity of a particle and its radius when falling in a liquid medium under the influence of gravity, the percentages of the particles of the different diameters in each sample were determined. The details of the construction of this apparatus and the procedure of analysis have been described by Goodhue and Smith (*14*).

The percentages of the particles in each sample below 2μ in diameter and between 2μ and 30μ and above are given in table 1. As approximately 28 percent of the commercial sample was composed of particles less than 2μ in diameter and about 60 percent of the particles in the reground samples were of this size, it is apparent that the regrinding operation increased the fineness of the commercial product. Unfortunately the sedimentation analysis was not sufficiently discriminating to differentiate between the particles less than 2μ in diameter. It is impossible to determine from these data whether the modifications in the grinding operation produced materials which differed significantly in their degrees of fineness.

TABLE 1.—*The size of the particles in the commercial and reground samples of acid lead arsenate*

Samples of acid lead arsenate	Proportion of particles of the following diameters, in microns—							
	Below 2	2–4	4–6	6–10	10–15	15–20	20–30	Above 30
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Commercial	28.2	36.0	15.1	7.3	5.3	2.3	2.3	3.5
Reground, No. 4	56.0	26.1	2.6	5.1	5.1	1.9	1.9	1.3
Reground, No. 5	61.4	15.0	3.5	5.1	10.0	3.4	1.1	.5
Reground, No. 6	69.0	15.1	2.6	3.2	4.2	3.5	1.2	1.2
Reground, No. 7	55.3	18.4	2.3	10.6	6.2	3.7	2.4	1.1
Reground, No. 8	58.0	17.2	2.8	6.2	7.9	3.8	2.8	1.3

It was very difficult to mix reground lead arsenate uniformly with soil because the particles of the insecticide tended to adhere tenaciously to the first grains of soil encountered. In the attempt to overcome this difficulty, the lead arsenate was shaken vigorously with dry sand until the grains of sand appeared to be uniformly covered before the sand and poison were applied to the soil. It is appreciated that adding the material in this condition to the soil might be equivalent to using acid lead arsenate with particles the size of those in the sand, but no better method for mixing a dry material intimately with soil has been developed. The different samples of lead arsenate thus diluted with dry sand were mixed with moist sassafras sandy loam at rates equivalent to 500, 1,000, 1,500, and 2,000 pounds of the poison per acre. Larvae were then introduced into each treatment.

The percentage of the larvae surviving in each treatment, out of the 200 introduced, the pounds of each sample estimated to be equivalent in insecticidal value to 1,000 pounds of the standard, and the summary of the analysis of variance in the larval survival are given in table 2.

The analysis of variance of survival showed that significant changes were produced by the modification in the rate of application of the arsenical and by the grinding. It was apparent, however, that the concentration of acid lead arsenate in the soil was the more important factor modifying the survival. When the lead arsenate was applied at the rate of 1,500 or 2,000 pounds per acre, no significant differences at odds of 99 to 1 could be detected between the survivals in the standard and the reground samples. It is apparent that when used at these rates there is sufficient acid lead arsenate in excess of the minimum required to mask the differences between the samples.

TABLE 2.—*Analysis of variance of larvae of the Japanese beetle surviving in 960 tests in which various amounts of acid lead arsenate containing different percentages of small particles* [1] *were tested as stomach poisons*

Sample of acid lead arsenate	Survival out of 200 larvae [2] in treatment proportional to indicated pounds of poison applied per acre				Estimate of test material equivalent to 1,000 pounds of the standard	Analysis of variance		
	500	1,000	1,500	2,000		Source of variation	Degrees of freedom	Mean square
	Percent	*Percent*	*Percent*	*Percent*	*Pounds*			
Commercial (standard)	68	49	12	3	----------	Between concentrations.	3	316.669**
Reground, No. 4	61.5	44	21	9.5	857±136			
Reground, No. 5	53	28.5	8	2	582± 67	Between materials	5	19.923**
Reground, No. 6	59	25.5	12	4	649± 60	Interaction	15	4.159**
Reground, No. 7	50	33.5	3	2.5	530±116	Error	936	.757
Reground, No. 8 [3]	37	16.5	4.5	5	Less than 500.	Total	959	--------

[1] See table 1 for analysis of sample for size of particle.
[2] Least significant difference for odds of 99 to 1, 10 percent.
[3] Ground with 2 percent of gum arabic.

There was no evidence that the grinding to which sample 4 was subjected significantly modified the insecticidal action. When the number of larvae surviving in the 1,000-pound treatment of the standard was taken as a measure of insecticidal effectiveness, it was found that the regrinding in samples 5, 6, 7, and 8 had greatly increased the effectiveness. The results suggest that 500 to 700 pounds of samples 5 and 6, 400 to 650 pounds of sample 7, and less than 500 pounds of sample 8 produced results against the larvae equivalent to 1,000 pounds of the unground commercial powder. It is possible that the increased effectiveness of sample 8 might be attributed to the gum arabic preventing the small particles from coalescing.

The reaction of rye to the various samples of acid lead arsenate in the soil was not significantly different, indicating that the solubility in the soil had not been greatly modified by the grinding.

Although the fineness of the particles was not so important a factor as the rate at which acid lead arsenate was applied, there is no doubt that the size of the particles did significantly change the insecticidal action.

INFLUENCE OF VARIATION IN ARSENIC CONTENT

Through the cooperation of P. A. Van der Meulen, at that time agent of the Bureau of Entomology, nine samples of acid lead arsenate which ranged in their arsenic oxide content from 29.79 to 33.77 percent, were prepared in 1934 to determine the influence of the arsenic oxide content on the insecticidal action in the soil. These materials were applied to sassafras sandy loam at the rates of 250, 500, 1,000, 1,500, and 2,000 pounds per acre. The percentages of survival of the larvae in the different treatments, the pounds of the different materials which were estimated to be equivalent in insecticidal value to 1,000 pounds of the standard, containing 33.77 percent arsenic oxide in this case, and the analysis of the variance are given in table 3.

TABLE 3.—*Analysis of variance of larval survival in 3,600 tests in which various amounts of acid lead arsenate containing different percentages of arsenic oxide were tested as stomach poisons for the larvae of the Japanese beetle*

Arsenic oxide in the samples (percent)	Survival out of 400 larvae [1] in treatment proportional to indicated pounds of poison applied per acre						Estimate of test material equivalent to 1,000 pounds of the standard (33.7 percent)	Analysis of variance		
	None	250	500	1,000	1,500	2,000		Source of variation	Degrees of freedom	Mean square
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pounds*			
33.77	83	64	46.75	11	5.5	3.5		Between concentrations	4	1,462.3212**
33.11		76	37	8.75	3	3.5	960 ± 57	Between materials	8	29.4159**
32.14		73.25	71.5	13.5	6.25	4	1,172 ±216	Interaction	32	10.1935**
31.93		72.5	49.25	11.25	3.75	2.25	1,017 ±202	Error	3,555	.8879
31.74		62	47.5	8.5	5.75	2.5	968 ± 52			
30.74		64.5	43	14.75	7.25	1.5	1,250 ±222	Total	3,599	
30.50		79.5	62	33.25	13	4.25	1,614 ±174			
30.00		74.25	51	16.75	6.75	1.75	1,288 ±172			
29.79		44	26.75	14.75	7	5.5	1,242 ±213			

[1] Least significant difference for odds of 99 to 1, 7.65 percent.

The analysis of variance in the larval survival showed that there were very significant differences in the insecticidal action of the samples containing various percentages of arsenic oxide. There was a definite tendency for the survival to increase as the arsenic oxide content was reduced from 33.77 to 29.79 percent, but the data are too variable and not sufficiently extensive definitely to establish this relationship. The actual differences in the mortality were not large. It is believed, therefore, that satisfactory results in controlling the larvae can be obtained with a commercial acid lead arsenate which contains the legal minimum of 30 percent of arsenic oxide.

Influence of Soluble Arsenic

In connection with the study of phosphate fertilizers on the effectiveness of acid lead arsenate as a stomach poison, the survival of larvae and the quantity of soluble arsenic in the soil were determined for each treatment. An analysis was made of the survival of the larvae in the treatments where acid lead arsenate was applied alone and with different phosphates, to determine whether there was any correlation between the survival and the soluble arsenic in the soil.

Only the 250- and 500-pound treatments were used in this analysis because the 1,000-, 1,500-, and 2,000-pound treatments gave very low larval survivals. This analysis showed that the quantity of soluble arsenic in the soil did not explain the differences in larval survival. It is evident that the concentration of soluble acid lead arsenate in the soil is not the most important factor modifying the larval survival.

As Swingle (*27*) has shown that the hydrogen-ion concentration of the midgut of third instars of the Japanese beetle is 9.5 and of the hindgut is about 8, and that these values are not greatly influenced by the hydrogen-ion concentration of the soil in which the larvae are living, it is apparent that the solubility of the acid lead arsenate in the soil having a hydrogen-ion concentration of 5.0, as was used in these tests, would be quite different from that in the digestive tract of the insect. It is evident from the previous analyses of variance that the total quantity of acid lead arsenate in the soil ingested by the larvae is the most important factor modifying the survival of larvae.

Influence of Lead Content

As Brinley (*4*) has demonstrated that lead salts were toxic to the eastern tent caterpillar (*Malacosoma americana* (F.)), and Stübinger (*26*) has shown the toxicity of lead compounds to bees and larvae of caddisflies, it appeared that the lead molecule in acid lead arsenate might have some influence on the insecticidal action. To determine the effect of the lead molecule on the larvae of the Japanese beetle it was necessary to remove it from the influence of the arsenic and to consider it in combination with acid radicals which usually would not be considered very toxic. Different lead salts were tested to determine their effectiveness in killing the larvae. The results obtained with the different materials and the analysis of the variance are given in table 4.

Table 4.—*Analysis of variance of larvae of the Japanese beetle surviving in 1,400 tests in which different lead compounds were tested as stomach poisons*

Test material	Survival out of 200 larvae [1] in treatment proportional to indicated pounds of material applied per acre						Analysis of variance		
	None	250	500	1,000	1,500	2,000	Source of variation	Degrees of freedom	Mean square
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*			
Acid lead arsenate (standard)	81	75	69.5	35	3	2.5	Between concentrations	4	51.4546**
Lead acetate	81	83.5	76	81	71.5	71	Between materials	6	110.7347**
Lead borate	81	81.5	86.5	66	51	50.5	Interaction	24	18.4371**
Lead carbonate	81	76.5	76	80	75.5	83	Error	1,365	1.1519
Lead chloride	81	80	81	77.5	76	67			
Lead fluoride	81	81.5	79	67	78	78	Total	1,399	
Lead hydrogen phosphate	81	72.5	60	62	67.5	67			

[1] Least significant difference for odds of 99 to 1, 12.5 percent.

Within the range of 250 to 2,000 pounds per acre none of these salts (lead acetate, lead borate, lead carbonate, lead chloride, lead fluoride, or lead hydrogen phosphate) caused a reduction in the number of larvae equivalent to that produced by 1,000 pounds of acid lead arsenate. When the survival in the untreated soil was compared with that in the different treatments, it was found that lead acetate, lead carbonate, and lead fluoride caused no significant change in the number of larvae surviving when applied at the rate of 2,000 pounds per acre. Lead chloride caused a significant reduction when applied at the rate of 2,000 pounds per acre, lead borate at 1,000 pounds, and lead hydrogen phosphate at 500 pounds.

The results appear to show that some of the lead salts are slightly toxic to the larvae. The insecticidal action, however, was not sufficient to be of value as a control measure. Later data will be presented to show that the arsenates of aluminum, barium, calcium, iron, magnesium, manganese, and zinc, arsenious oxide, arsenious sulfide, and paris green and its homologues have considerable toxicity. The indications are that the lead molecule contributes very little to the action of acid lead arsenate on the larvae, and it seems proper to associate the toxicity of this material with the arsenic molecule.

Persistence of Insecticidal Action

It is believed that any stomach-poison insecticide should be effective for more than one season in the soil to be of practical value. It is also recognized that the initial effectiveness of the insecticide is correlated with the presence of certain soluble salts in the soil and that the persistence of the insecticidal action is associated with the physical and chemical properties of the soil. In a problem of this nature, where the comparative value of acid lead arsenate and other materials are being studied, it is desirable to have some information on the persistence of the toxic action of acid lead arsenate under the same conditions as were used to evaluate the other materials as soil insecticides.

Acid lead arsenate was applied to sassafras sandy loam at rates equivalent to 500, 1,000, 1,500, and 2,000 pounds per acre. Larvae were introduced into the treated soil immediately after the application and at intervals up to 57 months, during which time the soils were exposed to weathering in the field. Each time an insecticidal test was made, the survival of larvae in these treatments was compared with that in soil to which acid lead arsenate had been freshly applied. The survival of the larvae in each treatment and the relative effectiveness of the material after it had been in the soil for different periods are given in table 5.

TABLE 5.—*The persistence of acid lead arsenate as a stomach poison against larvae of the Japanese beetle in sassafras sandy loam*

Exposure of test material in soil (months)	Larvae introduced	Survival of larvae in treatments equivalent to indicated pounds per acre										Minimum significant difference for odds of 99 to 1	Estimate of test material equivalent to 1,000 pounds of standard
		Acid lead arsenate applied at beginning of experiment					Freshly applied acid lead arsenate						
		None	500	1,000	1,500	2,000	None	500	1,000	1,500	2,000		
	No.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Lb.*
0	200	79	46.5	15	8.5	4	79	46.5	15	8.5	4	10.4	1,000± 89
6	100	88	80	35	19	13	84	69	32	15	2	19.0	1,094±214
7	200	89.5	68	36	27.5	8	79	46.5	15	8.5	4	10.4	1,821±121
12	100	88	60	42	11	5	84	69	32	15	2	19.0	1,161±124
13.5	200	97	74	43	30	4.5	91.5	48.5	6.5	2	.5	10.6	1,961±110
14.25	200	86	80.5	45.5	20	2	93	63.5	11	3	.5	8.9	1,750±108
18	100	92	80	70	32	20	84	69	32	15	2	19.0	1,500±137
24	100	88	52	56	27	9	84	69	32	15	2	19.0	1,414±180
26	200	85.5	76	53.5	19.5	1.5	90	36	5	1.5	1	9.8	1,903±143
30	300	86.6	88.3	69.6	47.6	34	71.6	54.6	30.6	15	12	17.6	Beyond 2,000
30	200	94	96	79.5	63.5	57	94	63	44.5	21.5	19.5	15.0	Beyond 2,000
32	200	91	79.5	41.5	24.5	5.5	81	46	2.5	2	0	10.0	Beyond 2,000
44	200	90	85	76.5	56	40.5	85.5	39.5	4.5	4.5	1	12.1	Beyond 2,000
45	200	94	75	53	25.5	2	96	47.5	8.5	.5	0	9.7	1,862±99
48	200	59.5	54	54.5	69	51.5	64	40.5	33	16.5	3	13.1	Beyond 2,000
57	200	65.5	58	56.6	47.5	53.5	79.5	37	22	8	4	12.3	Beyond 2,000

It was found that there was a progressive loss in the effectiveness of acid lead arsenate in sassafras sandy loam with the period of time the material was in the soil, although these differences did not appear to be statistically significant during the first year It is apparent that an application of 1,500 to 2,000 pounds per acre would be required to maintain an insecticidal value during a period of 18 to 24 months equivalent to 1,000 pounds of freshly applied acid lead arsenate, and that something more than 2,000 pounds would be required to obtain this action during a period of 30 months.

It was observed, however, that even after the material was in the soil for nearly 5 years the insecticidal action was not entirely dissipated. The effectiveness of the treatments was reduced to such an extent by this time that it is questionable whether they would be of great value as a control measure.

LEACHING OR FIXATION OF ACID LEAD ARSENATE IN SOIL

In the experiments discussed in the previous section the soil was in 5-inch pots during the whole period. As chemical analysis showed that there was little significant reduction in the total arsenic content during the 57 months, the decrease in the effectiveness cannot be attributed to the removal of the material by leaching. Under field conditions, particularly in some types of soil, it would be expected that the effectiveness of the material would be influenced to some extent by leaching.

In 1934, beds of soil in a greenhouse were treated to a depth of 6 inches with different quantities of acid lead arsenate with the object of developing a method for preventing the emergence of beetles in greenhouses growing roses during the winter months. Early in 1937 the experiment was discontinued and the plants and soil were removed from the beds. The soil was analyzed [4] to determine the quantities of arsenic remaining in the upper 6 inches of soil after the period of 3 years, the results being expressed as equivalent pounds of acid lead arsenate per acre, and then it was subjected to the regular biological assay to determine the effectiveness of the different treatments in killing larvae.

The survival of larvae in these treatments and in the same treatments freshly applied is given in table 6. The analysis of variance and the quantities of acid lead arsenate determined by chemical analysis and biological assay are also given in this table. The analysis of variance in survival showed that both the period of time the material was in the soil and the quantity applied were factors affecting the larval survival.

TABLE 6.—*Survival of larvae of the Japanese beetle and fixation and leaching of acid lead arsenate applied as a control in sassafras sandy loam in a rose house during a period of 3 years*

Quantity applied per acre to the beds to a depth of 6 inches (pounds)	Survival out of 200 larvae [1] in—		Quantity of acid lead arsenate per acre after 3 years				Analysis of variance of larval survival		
	Material freshly applied	Material in soil 3 years	Determined by chemical analysis	Estimated by biological assay	Loss from upper 6 inches by leaching	Apparently converted to a nontoxic form	Source of variation	Degrees of freedom	Mean square
	Percent	*Percent*	*Pounds*	*Pounds*	*Pounds*	*Pounds*			
None	91.5	100	----	----	----	----	Between treatments	6	169.6029**
500	65.5	66	500	490	0	10			
1,000	43	61.5	800	589	200	211	Between time intervals	1	256.5018**
1,500	12	56	1,200	711	300	489	Interaction	6	22.1184**
2,000	4	64.5	1,500	522	500	978	Error	546	.7390
2,500	1.5	38	1,700	1,080	800	620			
3,000	.5	21.5	2,100	1,347	900	753	Total	559	----

[1] Least significant difference for odds of 99 to 1, 9.9 percent.

The quantity of acid lead arsenate lost by leaching ranged from no lead arsenate with the 500-pound treatment to 900 pounds with the 3,000-pound application. The larger the quantity of arsenical applied, the greater was the relative loss by leaching. Nothing was lost from the treatment with 500 pounds, 20 percent from 1,000 pounds and from 1,500 pounds, 25 percent from 2,000 pounds, 32 percent from 2,500 pounds, and 30 percent from 3,000 pounds. In the rose beds in the greenhouse the average loss of acid lead arsenate by leaching in a period of 3 years was 25.7 percent.

The quantity of insecticidally active acid lead arsenate in the soil after 3 years, as estimated by biological assay, was significantly less

[4] These analyses were made by L. Koblitsky of the Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine.

than the quantity determined by chemical analysis. With the exception of the 500-pound treatment, the biological assay indicated that there was from 211 to 978 pounds per acre of the arsenical in an inactive form. With the initial treatment of 500 pounds, 2 percent was insecticidally inactive at the end of 3 years. The treatment with 1,000 pounds had 26.3 percent inactive; 1,500 pounds, 40.7 percent; 2,000 pounds, 65.2 percent; 2,500 pounds, 36.5 percent; and 3,000 pounds, 35.8 percent. In general, 39.2 percent of the acid lead arsenate remaining in the soil for 3 years was in an insecticidally inactive form. It is apparent that the chemical analysis of soil to determine total arsenic and lead may not be a reliable index of the possible effectiveness of the treatment in killing larvae of the Japanese beetle.

SUBSTITUTES FOR ACID LEAD ARSENATE

Although acid lead arsenate has generally been satisfactory for the control of larvae of the Japanese beetle in turf and for the elimination of them from blocks of established nursery plants, the detrimental effect of the material on some plants, the logical objections raised to the use of lead compounds and arsenicals in soil that may be used for growing food plants, and the cost of the treatment have stimulated the search for substitutes. Among the different classes of materials that have been investigated for this purpose are other inorganic arsenates, arsenious oxide, arsenious sulfide, paris green and its homologues, inorganic borates, inorganic fluorides, inorganic fluosilicates, derris, hellebore, mowrah meal, pyrethrum, fertilizers such as calcium cyanamid, ammonium sulfate, and acid phosphate, and lime.

Inorganic Arsenates

Preliminary small-scale experiments by Leach (*17*) indicated that basic lead arsenate, magnesium arsenate, and ferric arsenate were not toxic to larvae of the Japanese beetle. Leach reported that zinc arsenate and copper arsenate killed more slowly than acid lead arsenate and that calcium arsenate and acid lead arsenate appeared to be equivalent in toxicity.

In 1929 an investigation was undertaken to determine more definitely the possible value of different inorganic arsenates as substitutes for acid lead arsenate. The arsenates used in this investigation were analyzed by the Division of Insecticide Investigations of the Bureau of Entomology and Plant Quarantine. The composition of these materials is shown in table 7. The names used to designate the various samples are those used commercially and do not imply that definite, pure salts were available. In particular the two calcium arsenates are to be regarded as containing considerable quantities of the compounds named, but in admixture with such quantities of excess lime and calcium carbonate as to make their gross analyses practically identical. Scorodite, a natural ferrous arsenate, was not considered in this investigation because Snapp (*24*) found it to be practically worthless against the plum curculio (*Conotrachelus nenuphar* (Hbst.)), and Alden and Yeomans (*1*) reported poor results with it against the codling moth (*Carpocapsa pomonella* (L.)).

TABLE 7.—*Composition of the arsenates used in the study of soil insecticides for the larvae of the Japanese beetle*

Chemical	Moisture	Total arsenic oxide	Total arsenious oxide	Water-soluble arsenic oxide	Percentage of total base
	Percent	*Percent*	*Percent*	*Percent*	
Aluminum arsenate	7.28	33.71	0.69	0.72	39.82 as Al_2O_3.
Barium arsenate	.56	26.69	.10	2.38	65.55 as BaO.
Dicalcium arsenate	1.05	40.15	Trace	7.00	40.74 as CaO.
Tricalcium arsenate	1.13	40.64	.17	.20	40.57 as CaO.
Ferric arsenate	2.28	47.64	.16	1.19	33.66 as Fe_2O_3.
Basic lead arsenate	.32	22.09	.15	.17	72.59 as PbO.
Magnesium arsenate	3.79	32.84	.05	.61	39.58 as MgO.
Manganese arsenate	1.40	42.97	0	1.02	48.32 as MnO, 8.65 as CaO.
Zinc arsenate	.91	33.07	3.60	.34	57.94 as ZnO.

The different arsenates were applied to sassafras sandy loam at rates equivalent to 500, 1,000, 1,500, and 2,000 pounds per acre. The relative effcetiveness was determined immediately after the chemical was mixed with the soil and at intervals up to 60 months. The several analyses of variance of the survival of the larvae showed that significant differences in survival occurred with the change in the chemical composition, the rate of application, and the period the materials were in the soil. The survival of larvae in the different treatments with these arsenates and the quantities estimated to be equivalent to 1,000 pounds of freshly applied acid arsenate are given in table 8.

TABLE 8.—*Results of tests in which various quantities of different arsenates were used as stomach poisons for control of larvae of the Japanese beetle*

Chemical	Exposure of test material in soil	Larvae introduced	Survival of larvae in treatment equivalent to indicated pounds of material per acre: None	500	1,000	1,500	2,000	Survival in standard, freshly applied acid lead arsenate equivalent to 1,000 pounds per acre	Minimum significant difference for odds of 99 to 1	Estimate of test material equivalent to 1,000 pounds of the standard
	Mo.	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Lb.*
Aluminum arsenate	0	200	94	60.5	45.5	26	24	44.5	15	1,026±151
	6	100	84	63	40	30	18	32	19	1,400±471
	12	100	84	50	23	6	3	32	19	833±163
	18	100	84	62	40	25	16	32	19	1,267±278
	24	100	84	49	35	21	4	32	19	1,107±268
	30	200	94	73.5	56.5	43	37.5	44.5	15	1,444±287
	43	200	94	73	64	32.5	28.5	34.5	14.4	1,468±121
	48	200	79.5	74.5	51.5	37	35.5	33	13.5	Beyond 2,000
	60	200	79.5	57	62.5	53	48	22	13.2	Beyond 2,000
Barium arsenate	0	200	94	56	43.5	43	13.5	44.5	15	960±315
	6	100	84	68	27	6	1	32	19	939±119
	12	100	84	64	35	18	6	32	19	1,088±219
	18	100	84	75	50	41	26	32	19	1,800±286
	24	100	84	61	29	25	14	32	19	953±155
	30	200	94	75	67	46.5	40.5	44.5	15	1,667±510
	43	200	94	84.5	64	57.5	41.5	34.5	14.4	Beyond 2,000
	48	200	64	55	59.5	57.5	52.5	33	13.5	Beyond 2,000
	60	200	79.5	74.5	54.5	52	59	22	13.2	Beyond 2,000
Dicalcium arsenate	0	200	94	55.5	22	11.5	7.5	44.5	15	664± 91
	6	100	84	26	4	13	15	32	19	448± 85
	12	100	84	48	14	2	0	32	19	735±119
	18	100	84	53	15	11	9	32	19	776±110
	24	100	84	51	7	10	4	32	19	716± 91
	30	200	94	72	51	41.5	29	44.5	15	1,343±371
	43	200	94	77	62.5	52	28	34.5	14.4	Beyond 2,000
	48	200	64	60	72.5	76	53	33	13.5	Beyond 2,000
	60	200	79.5	53	59	53.5	34.5	22	13.2	Beyond 2,000

TABLE 8.—*Results of tests in which various quantities of different arsenates were used as stomach poisons for control of larvae of the Japanese beetle*—Continued

Chemical	Exposure of test material in soil	Larvae introduced	Survival of larvae in treatment equivalent to indicated pounds of material per acre					Survival in standard, freshly applied acid lead arsenate equivalent to 1,000 pounds per acre	Minimum significant difference for odds of 99 to 1	Estimate of test material equivalent to 1,000 pounds of the standard
			None	500	1,000	1,500	2,000			
	Mo.	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Lb.*
Tricalcium arsenate	0	100	84	50	5	3	0	32	19	700± 88
	6	100	84	57	26	9	14	32	19	903±152
	12	100	84	51	18	3	1	32	19	788±128
	18	100	84	60	20	8	6	32	19	850±112
	24	100	84	56	39	14	8	32	19	1,140±153
	31	200	94	77	54.5	39.5	31.5	34.5	14.4	1,813±410
	43	200	94	84.5	60.5	40	31.5	34.5	14.4	1,824±406
	48	200	64	70	58.5	54.5	52.5	33	13.5	Beyond 2,000
	60	200	79.5	55	63	54	42	22	13.2	Beyond 2,000
Ferric arsenate	0	200	94	73	43	28	7	44.5	15	975±133
	6	100	84	74	50	22	7	32	19	1,321±156
	12	100	84	80	46	18	9	32	19	1,250±147
	18	100	84	83	59	28	18	32	19	1,435±157
	24	100	84	59	58	43	36	32	19	Beyond 2,000
	30	200	94	84.5	79.5	69	55.5	44.5	15	Beyond 2,000
	44	200	90	85	78.5	72.5	57.5	34.5	14.5	Beyond 2,000
	48	200	64	70	63	55.5	60	33	13.5	Beyond 2,000
	60	200	79.5	52.5	59.5	55.5	49	22	13.2	Beyond 2,000
Basic lead arsenate	0	100	84	63	77	65	46	32	19	Beyond 2,000
	6	100	84	87	90	90	88	32	19	Beyond 2,000
	12	100	84	63	66	65	64	32	19	Beyond 2,000
	18	100	84	90	85	87	91	32	19	Beyond 2,000
	24	100	84	67	76	79	51	32	19	Beyond 2,000
	31	200	94	80.5	74	81.5	87	34.5	14.4	Beyond 2,000
	48	200	64	75.5	73.5	75	75.5	33	13.5	Beyond 2,000
	60	200	79.5	72.5	62.5	68.5	56	22	13.2	Beyond 2,000
Magnesium arsenate	0	100	84	38	11	16	27	32	19	611±139
	6	100	84	68	21	25	16	32	19	883± 98
	12	100	84	53	7	3	1	32	19	728± 88
	18	100	84	72	38	20	12	32	19	1,167±216
	24	100	84	53	35	13	5	32	19	1,068±168
	31	200	94	76	59.5	47	23	34.5	14.4	1,761±131
	44	200	90	81.5	66.5	45.5	24.5	34.5	14.5	1,762±200
	48	200	64	60.5	64	58	46	33	13.5	Beyond 2,000
	60	200	79.5	46.5	52.5	55.5	52	22	13.2	Beyond 2,000
Manganese arsenate	0	100	84	36	11	2	0	32	19	580±149
	6	100	84	80	42	27	15	32	19	1,333±294
	12	100	84	84	30	2	8	32	19	981± 94
	18	100	84	62	37	3	15	32	19	1,074±110
	24	100	84	52	39	1	6	32	19	1,092± 98
	31	200	94	85.5	69.5	39	27	34.5	14.4	1,688±249
	44	200	90	75	62	28.5	17	34.5	14.5	1,410±159
	48	200	64	56	56.5	45.5	50.5	33	13.5	Beyond 2,000
	60	200	79.5	64	71.5	51.5	44	22	13.2	Beyond 2,000
Zinc arsenate	0	100	84	54	29	14	18	32	19	940±196
	6	100	84	72	43	53	31	32	19	1,977±231
	12	100	84	52	27	16	6	32	19	900±188
	18	100	84	81	41	22	16	32	19	1,237±214
	24	100	84	53	38	29	13	32	19	1,333±490
	31	200	94	83	70.5	62	40.5	34.5	14.4	Beyond 2,000
	44	200	90	65	64.5	41.5	23	34.5	14.5	1,689±191
	48	200	64	73	69.5	62	38.5	33	13.5	Beyond 2,000
	60	200	79.5	58.5	52.5	60	41.5	22	13.2	Beyond 2,000

When freshly applied to the soil, it appeared that significantly less than 1,000 pounds of the arsenates of calcium, magnesium, and manganese were required to produce an effect equivalent to that of the standard; the quantities estimated for the arsenates of aluminum, barium, ferric iron, and zinc to produce this effect did not appear to be significantly different from 1,000 pounds. Basic lead arsenate was of low insecticidal value, the survival in the 2,000-pound treatment being so high that the quantity equivalent to 1,000 pounds of the standard could not be estimated.

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Although these data are not sufficiently extensive to permit definite determination of the periods the different arsenates remained effective as insecticides, it is possible to estimate these fairly well by considering how long an application of 2,000 pounds of the test material would be equivalent in insecticidal efficiency to 1,000 pounds of the freshly applied standard. For ferric arsenate this period was 2 years, for acid lead arsenate and zinc arsenate 2.5 years, and for the arsenates of aluminum, barium, calcium, magnesium, and manganese 3.5 to 4 years. It may be expected that there would be little difference in the persistence of the insecticidal action of the arsenates of ferric iron, acid lead, and zinc, but that the arsenates of aluminum, barium, calcium, magnesium, and manganese would be effective 1 or 2 years longer.

It is apparent from the insecticidal action that any of these arsenates, except basic lead arsenate, can be used to destroy larvae of the Japanese beetle in the soil. The effect of the arsenates on the larvae, however, appears to be closely associated with the effect of the materials on plants. Basic lead arsenate was practically nontoxic to larvae and caused no detrimental effect on plants when added to soil about their roots. The arsenates that proved toxic to the larvae were detrimental to plants in varying degrees. It was observed that rye grew normally in soil containing basic lead arsenate, that its growth was slightly retarded with the higher concentrations of acid lead arsenate, ferric arsenate, and barium arsenate, and that its growth was markedly retarded with all concentrations of the arsenates of aluminum, calcium, magnesium, manganese, and zinc. When the horticultural varieties of *Hydrangea macrophylla* DC. were planted in soil treated with the different arsenates, the plants in soil containing arsenates of aluminum, barium, calcium, magnesium, manganese, and zinc were dead or dying within 2 weeks. Three days after they were planted in the poisoned soil brown streaks, following the veins, appeared in the leaves. The effect of acid lead arsenate and ferric arsenate on the plants was much slower. The plants treated with these materials were not dead at the end of the growing season, but were poor in appearance when compared with untreated plants. Acid lead arsenate appeared to be the least detrimental of the larva-killing arsenates.

Subsequent to these tests acid lead arsenate was applied to the soil about the roots of many varieties of plants to determine whether the material could be used without causing serious damage to the plants. It was found[1] that seedling plants were more susceptible than mature plants, and that unhealthy plants were more easily injured than vigorous plants. Those plants that developed a strong taproot or deep penetrating roots generally grew better than those with delicate primary roots confined largely to the treated layer of soil. In general it was found that azalea, mountain-laurel, cherry-laurel, and rhododendron were retarded in growth, but that fir, box, retinospora, juniper, pine, yew, arborvitae, hemlock, and spruce could be grown satisfactorily in soil containing acid lead arsenate. The treatment had no adverse effect on 142 varieties of deciduous trees, shrubs, and vines, except clematis and *Hydrangea macrophylla*. Of the 140 varieties of

[1] FLEMING, WALTER E. EFFECT OF ACID LEAD ARSENATE ON DIFFERENT PLANTS WHEN APPLIED TO SOIL ABOUT THEIR ROOTS FOR DESTRUCTION OF LARVAE OF THE JAPANESE BEETLE. U. S. Bur. Ent. and Plant Quar. E-418, 32 pp. 1937. [Mimeographed.]

established herebaceous perennial plants, 81 had made a satisfactory growth at the end of 1 year, 37 were retarded, and 22 were killed. Rye and the clovers were the only cover crops which grew consistently well in soil treated with acid lead arsenate. Of 31 varieties of vegetable crops tested, only sweetpotato, potato, pepper, and tomato produced normal crops in treated soil. The application of acid lead arsenate to established turf in lawns and golf courses did not injure the finer grasses but tended to retard the development of annual bluegrass.

Although acid lead arsenate cannot be used promiscuously on all types of plants it is believed that it is the safest of the arsenates that can be used about the roots of growing plants to destroy larvae of the Japanese beetle.

Arsenious Oxide

According to Browne (*5*) Chinese farmers for many years have applied crude lump arsenic to soil to detroy earthworms. Jarvis (*15*) in 1916 reported work with white arsenic (arsenious oxide) in connection with the control of grubs attacking the roots of sugarcane. More recently Peirson and Nash (*22*) have recommended arsenious oxide for the control of white grubs in forest nurseries.

In 1929 an investigation was undertaken to determine the possible value of arsenious oxide for the control of larvae of the Japanese beetle. The material used in this work was not analyzed but was guaranteed by the manufacturer to be 98 to 100 percent pure.

Arsenious oxide was applied to sassafras sandy loam at rates equivalent to 500, 1,000, 1,500, and 2,000 pounds per acre. The effectiveness of these treatments, as compared with treatment with freshly applied acid lead arsenate, was determined immediately after the application and at intervals up to 91 months. The survival of larvae in the different tests during this period and the quantities estimated to be equivalent to 1,000 pounds of freshly applied acid lead arsenate are given in table 9.

Table 9.—*Results of tests with arsenious oxide as a stomach poison for control of larvae of the Japanese beetle*

Period of exposure of arsenious oxide in soil (months)	Larvae introduced	Survival of larvae in treatment equivalent to indicated pounds of material applied per acre to depth of 3 inches									Minimum significant difference for odds of 99 to 1	Estimate of test material equivalent to 1,000 pounds of standard
		Arsenious oxide					Acid lead arsenate (standard) freshly applied					
		None	500	1,000	1,500	2,000	500	1,000	1,500	2,000		
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pounds*
0	200	94	53	51.5	40	28	63	44.5	21.5	19.5	15	1,304±294
6	100	84	18	3	2	1	69	32	15	2	19	394± 71
12	100	84	29	4	1	1	69	32	15	2	19	473± 92
18	100	84	38	5	4	1	69	32	15	2	19	591±113
24	100	84	22	1	1	2	69	32	15	2	19	419± 77
30	200	94	37	14	12	7.5	63	44.5	21.5	19.5	15	434± 67
43	200	94	42	3.5	4	2.5	41.5	34.5	21	4.5	14.4	597± 73
48	200	79.5	44.5	13	4	5	37	22	8	4	13.2	857± 99
57	200	89	24	6.5	6.5	2.5	46.5	13.5	4	6	9	800±117
60	200	91.5	32.5	6	4	.5	61	4.5	2	1.5	8.5	1,375±131
69	200	89	53.5	14	2	3	46.5	13.5	4	6	9	1,021±148
72	200	91.5	34	7	1	.5	61	4.5	2	1.5	8.5	1,208±197
80	200	87.5	66.5	34.5	18	8	57.5	4.5	1	.5	9.1	Beyond 2,000
91	200	94.5	76	13.5	3.5	7.5	46	2.5	2	0	10	Beyond 2,000

In the first evaluation of arsenious oxide, immediately after application, the material did not appear to be significantly different from the standard material in the quantity required to produce insecticidal results. During the period from 6 to 30 months after application it appeared that an initial application of approximately 500 pounds of arsenious oxide produced results equivalent to those with 1,000 pounds of the freshly applied standard. This apparent discrepancy may be attributed to the difficulty of obtaining, at the time of application, a uniform dispersion in the soil of a heavy material such as arsenious oxide. The results indicate that subsequent handling and the natural movement of the material in the soil accomplished the desired result within 6 months. After being in the soil for 30 months arsenious oxide gradually lost its insecticidal effectiveness, but there was a period of 80 months before the survival in the 2,000-pound treatment was greater than that in the 1,000-pound treatment of freshly applied acid lead arsenate.

The results indicate that arsenious oxide is more effective than acid lead arsenate in killing larvae of the Japanese beetle in the soil and that the insecticidal properties are destroyed very slowly by the soil constituents. Unfortunately rye, grasses, and nursery plants appear to be intolerant of a larvicidal concentration of arsenious oxide in the soil. This detrimental action on the roots of plants practically precludes the use of the material as a substitute for acid lead arsenate in soil where plants are growing and restricts its use to those areas where the vegetation is not a factor.

Arsenious Sulfide

Arsenious sulfide occurs in nature in short rhomic prisms as orpiment, but no information was found on the possible use of this material as a stomach poison against soil-infesting insects. In 1935 some of this material was obtained so that its effectiveness against the larvae of the Japanese beetle could be determined. The purity of the sample is not known, but its identity was confirmed by the Division of Insecticide Investigations.

Arsenious sulfide was applied to sassafras sandy loam at rates ranging from 50 to 2,000 pounds per acre and tested in the usual manner in comparison with acid lead arsenate. The biological assay of the material was made immediately after application and after the material had been 7 and 31 months, respectively, in the soil. The results of these tests are given in table 10.

During the first year that arsenious sulfide was in the soil it appeared to be of about the same order of effectiveness as arsenious oxide. After being in the soil for about 2½ years, however, it appeared that an initial application of $1,469 \pm 152$ pounds would have been required to be equivalent at this time to 1,000 pounds of freshly applied acid lead arsenate. As the equivalent of arsenious oxide for this period was 434 ± 67, it is evident that arsenious sulfide lost its insecticidal value more rapidly in the soil than did arsenious oxide.

Arsenious sulfide appeared to be very detrimental to the growth of plants. Rye made only a feeble growth in soil containing as little as 50 pounds of the material per acre. This injurious action on plants definitely limits its usefulness as a soil insecticide.

TABLE 10.—*Results of tests with arsenious sulfide as a stomach poison for control of larvae of the Japanese beetle*

Period of exposure of arsenious sulfide in soil (months)	Larvae introduced	Survival of larvae in treatment equivalent to indicated pounds of material applied per acre to a depth of 3 inches — Arsenious sulfide									
		None	50	100	150	200	250	500	1,000	1,500	2,000
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0	400	64.3	62	47.5	40	26.5	18.8	7.8	7.5	8.5	6
7	200	80	63	54.5	46.5	33	28.5	6.5	1.5	2.5	2
31	200	79	79	78	75.5	75.5	68	34	19.5	3.5	0

Period of exposure of arsenious sulfide in soil (months)	Larvae introduced	Survival of larvae in treatment equivalent to indicated pounds of material applied per acre to a depth of 3 inches—Continued — Acid lead arsenate (standard) freshly applied					Minimum significant difference for odds of 99 to 1	Estimate of test material equivalent to 1,000 pounds of standard
		250	500	1,000	1,500	2,000		
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pounds*
0	400	65	61.5	11.3	4	2.5	6.8	420± 72
7	200	47	19.5	12	3	2	9.1	438± 50
31	200	----------	57.5	4.5	1	.5	9.1	1,469±152

PARIS GREEN AND ITS HOMOLOGUES

During 1934 an investigation was made to determine the possible value of paris green and certain of its homologues as stomach poisons against the larvae of the Japanese beetle. The results obtained with these materials as stomach poisons and repellents against the adult beetle have been published (*12*).

The paris green used in this work was purchased on the open market and was analyzed by L. Koblitsky of the Division of Insecticide Investigations. The homologues of paris green were prepared and analyzed by F. E. Dearborn of that Division. The description of the preparation and the method of analyzing the homologues of paris green has been published by Dearborn (*9*, *10*). The composition of the materials which were used in the present study is given in table 11.

TABLE 11.—*Paris green and homologous materials investigated, and their arsenious oxide and cupric oxide content*

Material	Total As_2O_3 in dry material	CuO in dry material	Water-soluble As_2O_3 in dry material
	Percent	*Percent*	*Percent*
Paris green	35.52	29.90	1.94
Copper lauroarsenite	43.65	24.23	1.94
Copper palmitoarsenite	38.59	22.10	4.88
Copper oleoarsenite	35.45	19.89	2.61
Copper stearoarsenite	31.38	20.72	4.29

Paris green and its homologues were applied to sassafras sandy loam at the rates of 250, 500, 1,000, 1,500, and 2,000 pounds per acre and tested in comparison with acid lead arsenate. The survival of larvae in each treatment and the pounds of each material estimated to be equivalent to 1,000 pounds of acid lead arsenate are given in table 12.

TABLE 12.—*Results of tests with paris green and its homologues as stomach poisons for control of larvae of the Japanese beetle*

Test material	Survival out of 200 larvae [1] in treatment proportional to indicated pounds of material applied per acre						Estimate of test material equivalent to 1,000 pounds of acid lead arsenate
	None	250	500	1,000	1,500	2,000	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pounds*
Acid lead arsenate	94	75.5	63.5	26.5	10	0.5	
Paris green		47	14	7.5	6	0	405± 38
Copper lauroarsenite		77.5	57.5	35.5	21.5	11	1,321±191
Copper palmitoarsenite		78.5	73.5	38	32	7.5	1,612± 94
Copper oleoarsenite		25.5	23.5	17.5	12	4.5	246± 23
Copper stearoarsenite		73.5	75.5	26	11.5	10.5	995± 64

[1] Least significant difference for odds of 99 to 1, 11.6 percent.

Paris green and copper oleoarsenite appeared to be significantly more effective than acid lead arsenate; copper stearoarsenite appeared to be equivalent, and copper lauroarsenite and copper palmitoarsenite, less effective than acid lead arsenate against the larvae. All these materials were detrimental to the growth of rye. This injurious action on plants definitely limits the use of paris green and its homologues as soil insecticides.

INORGANIC BORATES

In 1935 an investigation was made of some inorganic borates to determine their value as substitutes for acid lead arsenate for control of Japanese beetle larvae. The materials were prepared by the manufacturer for this work. As the purity of the materials was not determined by analysis, the names used to designate the various materials should be considered to mean that probably a high percentage of the compound was present but possibly in admixture with other salts. It is not implied that definitely pure salts were available for this work.

The different borates of calcium, lead, magnesium, nickel, sodium, strontium, and zinc were applied to sassafras sandy loam at rates ranging from 250 to 2,000 pounds per acre. The survival of larvae in each treatment is given in table 13.

TABLE 13.—*Results of tests with inorganic borates as stomach poisons for control of larvae of the Japanese beetle*

Test material	Survival out of 400 larvae [1] in treatment proportional to indicated pounds of material per acre						Estimate of test material equivalent to 1,000 pounds of acid lead arsenate
	None	250	500	1,000	1,500	2,000	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pounds*
Acid lead arsenate	81	76.3	61.3	19	1.8	3	
Calcium borate		73.5	79.3	60.3	45.3	38.3	Beyond 2,000
Lead borate		81.8	86.8	66	51	50.5	
Magnesium borate		74.3	70.3	50	43.3	36.3	
Nickel borate		70.5	67.5	43.5	38.3	34.3	
Sodium borate		63.8	70.5	52.5	38	30.5	
Strontium borate		78.3	63.8	50.8	39.8	36.3	
Zinc borate		73.3	71.8	48.5	44.3	37	

[1] Minimum significant difference for odds of 99 to 1, 11.6 percent.

It is evident that the borates possessed some larvicidal value. When the survival in the untreated soil was compared with that in tho various treatments, it was found that a significant reduction in the number of larvae was obtained with 500 pounds of the borates of nickel and strontium and with 1,000 pounds of the borates of calcium, lead, magnesium, sodium, and zinc. With the possible exception of lead borate, there appeared to be no significant differences in the insecticidal action of the borates when applied at the rates of 1,000, 1,500, and 2,000 pounds per acre. The survival of larvae in these dosages of lead borate was significantly greater than that obtained with some of the other borates. The evidence is not conclusive, however, that this compound should be considered to be less effective.

The borates of calcium, lead, magnesium, nickel, sodium, strontium, and zinc were not sufficiently toxic to the larvae of the Japanese beetle to be of value in the control of this insect. In addition, these materials in the soil were detrimental to the growth of rye. The results indicate that these borates are not satisfactory substitutes for acid lead arsenate.

Inorganic Fluorides

Preliminary small-scale tests during 1925 and 1926 gave indications that some of the inorganic fluorides might be of value as stomach poisons against the larvae of the Japanese beetle. In 1931 an investigation was undertaken to establish more definitely their value as such.

The purity of the natural cryolite was specified by the manufacturer, and that of the other fluorides was determined by the Bureau of Chemistry of the United States Department of Agriculture in 1926. The materials used in this work and their purity are given in the following list:

Material	*Percentage composition*
Aluminum fluoride (AlF_3)	65. 7
Sodium fluoride (NaF)	1. 7
Moisture and undetermined	32. 6
Barium fluoride (BaF_2)	99. 1
Moisture and undetermined	. 9
Calcium fluoride (CaF_2)	94. 9
Magnesium fluoride (MgF)	1. 6
Moisture and undetermined	3. 5
Copper fluoride ($CuF_2.2\ H_2O$)	80. 2
Copper sulfate ($CuSO_4.5\ H_2O$)	21. 5
Lead fluoride (PbF_2)	99. 8
Moisture and undetermined	. 2
Magnesium fluoride (MgF_2)	89. 1
Calcium fluoride (CaF_2)	5. 1
Moisture and undetermined	5. 8
Natural cryolite ($AlF_3.3\ NaF$)	92. 0
Moisture and undetermined	8. 0
Strontium fluoride (SrF_2)	99. 2
Moisture and undetermined	. 8
Zinc fluoride (ZnF_2)	93. 4
Moisture and undetermined	6. 6

The different fluorides were applied to sassafras sandy loam at the rates of 250, 500, 1,000 1,500, and 2,000 pounds per acre. The survival of larvae in each treatment is given in table 14.

TABLE 14.—*Results of tests with inorganic fluorides as stomach poisons for control of larvae of the Japanese beetle*

Test material	Survival out of 200 larvae [1] in treatment proportional to indicated pounds of material per acre						Estimate of test material equivalent to 1,000 pounds of acid lead arsenate
	None	250	500	1,000	1,500	2,000	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pounds*
Acid lead arsenate	81	75	69.5	35	3	2.5	
Aluminum fluoride		80	81	77.5	76	67	Beyond 2,000.
Barium fluoride		83.5	86	83.5	93	76	Beyond 2,000.
Calcium fluoride		83.5	83	79	71	78.5	Beyond 2,000.
Copper fluoride		83.5	76	81	71.5	71	Beyond 2,000.
Lead fluoride		81.5	79	67	78	78	Beyond 2,000.
Magnesium fluoride		79	79	83	67	75.5	Beyond 2,000.
Strontium fluoride		76.5	76	80	75.5	83	Beyond 2,000.
Zinc fluoride		70.5	70	68.5	56	34	1,977±143.
Natural cryolite		80.5	81	74	81.5	87	Beyond 2,000.

[1] Least significant difference for odds of 99 to 1, 11.7 percent.

The fluorides in the soil appeared to have no detrimental effect on the growth of rye. The low toxic value of the aluminum and zinc fluorides and the apparent lack of toxicity of the other fluorides to larvae of the Japanese beetle indicate that these materials cannot be considered as satisfactory substitutes for acid lead arsenate.

INORGANIC FLUOSILICATES

Preliminary tests by the writer in 1925, by Lipp (*19*) in 1925–26, and later by Metzger (*20*) indicated that some of the inorganic fluosilicates might be of value against the larvae of the Japanese beetle in the soil. In 1931 an investigation was undertaken to determine more definitely the possible value of these materials for this purpose.

As with some of the other materials, the designations of the different fluosilicates do not imply that definitely pure salts were available for this work. The purity of the magnesium fluosilicate was specified by the manufacturer, and that of the other fluosilicates was determined by the Bureau of Chemistry in 1926. The materials used in this study and their purity are as follows:

Material	*Percentage composition*
Barium fluosilicate ($BaSiF_6$)	98.8
Moisture and undetermined	1.2
Calcium fluosilicate ($CaSiF_6$)	29.0
Calcium fluoride (CaF_2)	47.6
Silica (SiO_2)	10.4
Moisture and undetermined	13.0
Magnesium fluosilicate ($MgSiF_6$)	98.0
Moisture and undetermined	2.0
Potassium fluosilicate (K_2SiF_6)	99.5
Moisture and undetermined	.5
Sodium fluosilicate (Na_2SiF_6)	99.6
Moisture and undetermined	.4

The different fluosilicates were applied to sassafras sandy loam at the rates of 500, 1,000, 1,500, and 2,000 pounds per acre and tested in comparison with acid lead arsenate. The effectiveness of the different treatments was determined immediately after application and after the materials had been weathering in the soil in the field for 6 months. The survival of the larvae and the pounds of each material estimated to be equivalent to 1,000 pounds of freshly applied acid lead arsenate are given in table 15.

TABLE 15.—*Results of tests with inorganic fluosilicates as stomach poisons for the control of larvae of the Japanese beetle*

Chemical	Period of exposure of fluosilicates in soil	Survival out of 100 larvae in treatment equivalent to indicated pounds of material applied per acre to a depth of 3 inches					Minimum significant difference for odds of 99 to 1	Estimate of test material equivalent to 1,000 pounds of acid lead arsenate
		None	500	1,000	1,500	2,000		
	Months	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pounds*
Barium fluosilicate	0	84	66	30	7	1	15.6	972±115
	6	88	84	75	71	45	20.3	Beyond 2,000
Calcium fluosilicate	0	84	78	78	76	69	15.6	Beyond 2,000
Magnesium fluosilicate	0	84	62	51	13	12	15.6	1,250±88
	6	88	95	89	62	49	20.3	Beyond 2,000
Potassium fluosilicate	0	84	72	24	7	3	15.6	917±81
	6	88	81	87	77	61	20.3	Beyond 2,000
Sodium fluosilicate	0	84	59	6	2	1	15.6	755±64
	6	88	87	78	75	65	20.3	Beyond 2,000
Checks:								
Acid lead arsenate freshly applied at beginning of experiment	0	84	69	32	15	2		
Acid lead arsenate freshly applied when the second test was made of the fluosilicates 6 months later	0	88	80	35	19	13		

When freshly applied, sodium fluosilicate was significantly more effective than acid lead arsenate, the fluosilicates of barium and potassium appeared to be equivalent, and magnesium fluosilicate significantly less effective. Calcium fluosilicate was practically nontoxic within the range of treatments tested. After being in the soil for 6 months the fluosilicates were of no value as stomach poisons against the larvae. It is apparent that leaching or the rapid decomposition of these fluosilicates in the soil to less effective compounds makes them unsatisfactory as substitutes for acid lead arsenate.

DERRIS, HELLEBORE, MOWRAH MEAL, PYRETHRUM, AND ROTENONE

Mowrah meal, the ground presscake left after the removal of oil from seeds of *Bassia longifolio* L., the active principle of which appears to be mowrin, was reported by Carlos (*6*) and Johnston (*16*) to be very effective in killing earthworms in the soil. Weston (*30*) suggested that it might be of value for the control of grubs in the soil. Lea (*18*) reported that young strawberry plants may be protected from the attacks of *Rhadinosomus lacordairei* Pasc. and *Rhinaria perdix* Pasc. by placing hellebore in the holes in which they are planted. Cook and Hutchison (*8*) found hellebore effective against fly larvae in horse manure. No reference to the use of derris, pyrethrum, or rotenone against soil-infesting insects was found in recent literature.

An investigation was undertaken to determine the possible values of these materials as poisons against larvae of the Japanese beetle. No information is available on the purity of the samples of pyrethrum, hellebore, and mowrah meal used in these tests. The derris contained 4 percent of rotenone, and the rotenone was of the c. p. grade and the technical grade, the latter 90 percent pure.

The materials were applied to sassafras sandy loam at the rates of 500, 1,000, 2,000, 4,000, and 8,000 pounds per acre and tested in comparison with acid lead arsenate. The number of larvae surviving in each treatment is given in table 16.

TABLE 16.—*Results of tests with derris, hellebore, mowrah meal, pyrethrum, and rotenone as poisons for control of larvae of the Japanese beetle*

Test material	Survival out of 200 larvae [1] in treatment proportional to indicated pounds of material per acre						Estimate of test material equivalent to 1,000 pounds of acid lead arsenate
	None	500	1,000	2,000	4,000	8,000	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pounds*
Acid lead arsenate	85	54	9	4.5	0	0	
Derris		74	64	58	49.5	21.5	Beyond 8,000
Derris [2]		88.5	84	80.5	78	75.5	
Hellebore		77	79	73	77	66.5	
Mowrah meal		78.5	69	60	63.5	56	
Pyrethrum		80	83.5	75.5	74	27	
Rotenone, c. p.		80.5	80.5	79.5	78	75.5	
Rotenone, technical		85.5	70	69	78.5	79	

[1] Least significant difference for odds of 99 to 1, 12 percent.
[2] Plus the equivalent of 2,000 pounds of lime per acre with each treatment.

When the number of larvae surviving in the untreated soil was compared with the survival in the different treatments, it was apparent that some of the materials were at least mildly toxic to the larvae. One thousand pounds of derris caused a significant reduction in the larval population. As even 8,000 pounds of rotenone, one of the active ingredients of derris, caused no significant reduction in the larval population, the results suggest that the rotenone content of the derris is not an important factor in the insecticidal action of derris as a stomach poison against the larvae. The addition of hydrated lime with the derris apparently destroyed its insecticidal action. It is known that rotenone oxidizes in the presence of alkali with a resultant reduction in toxicity. This would indicate that it is unwise to employ supplementary materials of an alkaline reaction with derris. Mowrah meal caused a significant reduction in the number of larvae when applied at the rate of 1,000 pounds per acre, as did hellebore and pyrethrum when used at the rate of 8,000 pounds per acre.

As the quantity of derris, hellebore, mowrah meal, or pyrethrum required to be equivalent in insecticidal action to 1,000 pounds of acid lead arsenate appeared to be in excess of 8,000 pounds per acre, it is apparent that these materials have no practical value as substitutes for acid lead arsenate in the soil.

FERTILIZERS AND SOIL CONDITIONERS

Different fertilizers have been reported from time to time as reducing the population of soil-inhabiting insects. In 1916 Cook and Hutchison (*8*) indicated that calcium cyanamid was effective against fly larvae in horse manure. Muir and Swezey (*21, p. 301*) reported that the application of calcium cyanamid gave excellent results against soil-inhabiting insects. Several times it has been reported from nonentomological sources that heavy applications of this material reduced white grub injury in pasture lands. Some growers of roses in greenhouses believe that applications of hydrated lime destroy the larvae of the Japanese beetle in the beds. Metzger (*20*) found that top-dressing rose beds with hydrated lime at the rate of 3,000 pounds per acre apparently reduced the emergence of adult Japanese beetles, about 77 percent, but the number remaining was sufficient to cause considerable damage. An investigation was accordingly undertaken to determine more definitely the value of different fertilizers and dressings for the control of larvae of the Japanese beetle in the soil. The survival of larvae in the different treatments and comparable tests with acid lead arsenate are given in table 17.

ACID PHOSPHATE

Acid phosphate was applied to sassafras sandy loam at the rates of 1,000, 2,000, 4,000, and 8,000 pounds per acre and tested in comparison with acid lead arsenate. When the survival in the untreated soil is compared with that in soil to which 8,000 pounds of acid phosphate was applied, it is apparent that the acid phosphate caused no significant reduction in the number of larvae, indicating that this material has little toxicity to them.

AMMONIUM SULFATE

Ammonium sulfate was applied at the same rates and tested at the same time as the acid phosphate. There was considerable variation in the larval mortality in the soil to which ammonium sulfate was applied. When the survivals in the different dosages were compared with that in untreated soil, it appeared that a significant reduction had been obtained with the 1,000- and 4,000-pound and possibly with the 8,000-pound applications and that no significant change had occurred with the 2,000-pound treatment. It is supected that these variations might be attributed to abnormal experimental variation. At least the results fail to demonstrate conclusively that ammonium sulfate has a detrimental influence on the larvae in the soil.

LIMESTONE

Ground limestone was applied and tested in the same manner. The results of these tests indicate that applications of ground limestone up to 8,000 pounds per acre have no detrimental effect on the larvae.

HYDRATED LIME

Hydrated lime was applied to sassafras sandy loam at rates ranging from 1,500 to 25,000 pounds per acre. When the survival in the soil to which hydrated lime was applied was compared with that in the untreated soil, it was found that applications of 10,000 or more pounds per acre of hydrated lime caused a consistent significant reduction in the numbers of surviving larvae. The action of the lower concentrations of hydrated lime was not consistent. The results indicate that hydrated lime may be of slight value in reducing the number of larvae in the soil, but an application of even 25,000 pounds of hydrated lime appeared to be of little value as a control measure. More larvae were killed with 250 pounds of acid lead arsenate than with this application

TABLE 17.—*Results of tests with different fertilizers and soil conditioners as stomach poisons for control of the larvae of the Japanese beetle*

Material	Larvae introduced	Survival of larvae in treatment equivalent to indicated pounds of material applied per acre to depth of 3 inches														Minimum significant difference for odds of 99 to 1	Estimate of fertilizer equivalent to 1,000 pounds of acid lead arsenate
		None	250	500	1,000	1,500	2,000	4,000	6,000	8,000	10,000	15,000	16,000	20,000	25,000		
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pounds*
Acid phosphate	200	86	-----	-----	76	-----	78	73.5	-----	79.5	-----	-----	-----	-----	-----	11.8	Beyond 8,000
Ammonium sulfate	200	86	-----	-----	68	-----	76	69	-----	74	-----	-----	-----	-----	-----	11.8	
Limestone	200	86	-----	-----	90	-----	78.5	91.5	-----	90.5	-----	-----	-----	-----	-----	11.8	
Check for the above 3 materials, acid lead arsenate freshly applied	200	86	80	27.5	22.5	3.5	2.5	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Hydrated lime	400	79.5	-----	-----	-----	86.5	61	65.8	73.5	-----	72	69.5	-----	69	71	7	Beyond 25,000
Check for hydrated lime, acid lead arsenate freshly applied	400	79.5	60.3	43.8	22.5	6.3	2.5	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Calcium cyanamid	400	81	75	74	77.8	69.3	64.8	38.3	-----	16	-----	-----	8.8	-----	-----	7.5	7,462±695
Check for calcium cyanamid, acid lead arsenate freshly applied	400	81	76.3	61.3	19	1.8	3	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

of hydrated lime. Hydrated lime appears of no value as a substitute for acid lead arsenate.

CALCIUM CYANAMID

Commercial calcium cyanamid, claimed by the manufacturer to contain 22 percent of nitrogen and 70 percent of hydrated lime, was applied at rates ranging from 250 to 16,000 pounds per acre. When the survival in the untreated soil was compared with that in the soil containing calcium cyanamid, it was apparent that an application of 1,500 pounds or more of the calcium cyanamid significantly reduced the larval population. These results suggest that calcium cyanamid might be of value in reducing the density of the larvae in pastures and in other large areas of grass where it is not desirable to use arsenicals. As a treatment of 7,000 to 8,000 pounds of calcium cyanamid per acre was required to produce an insecticidal action equivalent to 1,000 pounds of acid lead arsenate, it is apparent that this material is of too low toxic value to be used as a substitute for acid lead arsenate.

SUMMARY AND CONCLUSION

Experiments were conducted under controlled conditions to determine the value of different arsenicals, fluorine compounds, boron compounds, derris, pyrethrum, hellebore, mowrah meal, and various fertilizers and soil conditioners as substitutes for acid lead arsenate against the larvae of the Japanese beetle in the soil. In the search for new insecticides the determination of the relative effectiveness of many materials is more important than isolated determinations on the action of individual compounds.

The action of a stomach poison in the soil against this insect is the resultant of many complex factors, being influenced by the development, activity, and susceptibility of the larvae, the nature and the concentration of the material, and the physical and chemical characteristics of the soil.

The work with the different materials in the laboratory was conducted in 5-inch earthen pots in chambers maintained at 80° to 85° F. By maintaining this temperature it was possible to complete tests in 2 weeks, whereas at 55° to 60° 6 to 8 weeks would have been required. Tests were also made in beds in a greenhouse.

The procedure for conducting the tests to determine the value of a material as a stomach poison against the larvae is fundamentally the same as that used in the biological assay of drugs and disinfectants. The mortality produced by different concentrations of the test material and the standard (acid lead arsenate) was determined experimentally each time under the same conditions. As the larvae obtained at different seasons and in different fields were not constant in their susceptibility to the standard, each assay of a material was based on parallel tests between the standard and the test material. The details of the procedure are described in the text.

In each series of tests there were usually four independent sources of variation, namely, the chemical composition of the materials, the rate of application, the period the materials were in the soil before testing, and the random or experimental error. The analysis of variance was found to be the most satisfactory procedure for determining the significance of the difference in mortality caused by these factors. The pounds of the test material which were equivalent to

1,000 pounds of freshly applied acid lead arsenate were estimated by interpolation of the larval survivals.

Freshly applied acid lead arsenate was used as a standard of insecticidal effectiveness in these tests. Some consideration was given to the effect of the physical and chemical properties of this compound on the insecticidal action and to the persistence of the larvicidal action under experimental conditions. It was found that regrinding the commercial material, alone or with gum arabic, significantly increased the percentage of the particles in the samples less than 2 μ in diameter and enhanced the effectiveness of the material as an insecticide. The total arsenic oxide content of the material did not appear to be a major limiting factor, provided the material contained not less than 30 percent of arsenic oxide. Only a low correlation was found between the quantity of soluble arsenic in the soil and the larval mortality. The lead content of the compound did not appear to be a factor in the insecticidal action.

Under the experimental conditions, the effectiveness of acid lead arsenate decreased so slowly during the first year that no significant difference could be determined by means of the biological assay. After this period the loss in insecticidal action was accelerated. This loss in insecticidal value is attributed to leaching from the soil and to the conversion of the arsenical into a nontoxic form by reaction with the soil constituents. The determination of the arsenic and lead in the soil by chemical analysis may not be a reliable index of the possible effectiveness of the treatment in killing larvae because so far it has not been possible to separate the effective from the inactive arsenic in the soil.

When freshly applied, the arsenates of calcium, magnesium, and manganese appeared to be more effective against the larvae than acid lead arsenate; the arsenates of aluminum, barium, ferric iron, and zinc appeared to be equivalent to acid lead arsenate; and basic lead arsenate was of no insecticidal value. The effectiveness of acid lead arsenate, ferric arsenate, and zinc arsenate decreased rapidly after these arsenicals had been in the soil for about 2 to 3 years. The effectiveness of the other arsenates appeared to last 1 or 2 years longer, but with the exception of basic lead arsenate these were more toxic to plants growing in the soil than was acid lead arsenate.

Arsenious oxide was more toxic to the larvae than was acid lead arsenate and it was affected very slowly by the soil. The detrimental effect of this material on plants definitely limits its usefulness as a stomach poison in the soil.

Arsenious sulfide appeared to be about the same as arsenious oxide in its action on the larvae and resistance to change in the soil. Its high toxicity to plants limits its usefulness.

Paris green and certain of its homologues, namely, copper oleoarsenite, copper lauroarsenite, copper palmitoarsenite, and copper stearoarsenite, could be substituted for acid lead arsenate to destroy larvae of the Japanese beetle. The application of these materials, however, is also limited by their toxicity to plants.

The inorganic borates of calcium, lead, magnesium, nickel, sodium, strontium, and zinc were not sufficiently toxic to the larvae to be of value as stomach poisons.

The inorganic fluorides of aluminum, barium, calcium, copper, lead, magnesium, strontium, and zinc, and natural cryolite were not toxic to the larvae.

The fluosilicates of barium, magnesium, potassium, and sodium were equivalent to, or even better than, acid lead arsenate when freshly applied, but their rapid decomposition in the soil to nontoxic compounds or loss of their insecticidal constituents by leaching makes them unsuitable as substitutes for acid lead arsenate. Calcium fluosilicate was nontoxic when freshly applied to the soil.

Derris, hellebore, mowrah meal, and pyrethrum were so slightly toxic to the larvae as to be of little practical value for the control of this insect in the soil. Rotenone did not appear to be the ingredient in derris which was toxic to the larvae. The slight insecticidal action of derris was destroyed by adding lime to the soil.

Different fertilizers and soil conditioners such as acid phosphate, ammonium sulfate, ground limestone, hydrated lime, and calcium cyanamid were slightly toxic to larvae of the Japanese beetle. It may be possible to reduce the larval population by heavy applications of these materials, but it is questionable whether the decrease in the number of larvae would usually be sufficient to afford protection to plants.

In conclusion, the results of this investigation with different arsenicals, boron compounds, fluorine compounds, plant materials such as derris and pyrethrum, and different fertilizers show that some of these materials may be substituted for acid lead arsenate to kill larvae of the Japanese beetle in the soil under those conditions where the effect of the material on the vegetation is not a limiting factor. When the susceptibility of the plants growing in the soil to chemical injury is a factor, however, acid lead arsenate appeared to be the safest of the larva-killing materials for application to the soil.

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